combined with platinum chemotherapy is specifically indicated for treatment of non-squamous non-small cell lung cancer (non-sq NSCLC). Pemetrexed is a folate-analog metabolic inhibitor that disrupts folate-dependent processes essential for cell replication. Pemetrexed inhibits thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT), which are folate-dependent enzymes involved in the de novo biosynthesis of thymidine and purine nucleotides. Folate receptor alpha (FRalpha) is a folate/antifolate transporter protein that is overexpressed by a number of epithelial tumors. The purpose of this study is to identify proteomic biomarkers predictive of response to pemetrexed-based chemotherapy in non-sq NSCLC.

Methods: Patients with advanced non-sq NSCLC who received pemetrexed-based chemotherapy at West Virginia University from 2009 to 2014 were retrospectively identified. Formalin-fixed, paraffin-embedded tumor biopsies were laser microdissected, solubilized, enzymatically digested and subjected to quantitative proteomic analysis. A multiplexed, selected reaction monitoring (SRM) mass spectrometry (MS) assay was used to determine the absolute levels of 46 different candidate proteomic markers, including those in the folate receptor pathway. TS analysis was also performed by IHC. The Kaplan-Meier method and log-rank test were used in statistical analysis of overall survival (OS) and progression-free survival (PFS).

Results: The 74 patients included in the study had a median follow-up of 26 months, a median OS of 16.6 months (95%CI: 11.6 - 43.4), and a median PFS of 9.61 months (95%CI: 8.43, 12.98). There were 65 patients who received pemetrexed-based regimen as a first line therapy and 9 patients as subsequent salvage treatment. In a comparison between patients who survived >24 months and < 8 months, there were no significant differences between the two groups in terms of sex, age, ECOG performance status, TNM stage at diagnosis, and smoking history. Among the 37 patients with sufficient tumor specimens available for multiplexed proteomic analysis, 30 biomarkers were detected with varying levels of expression. Sixteen additional biomarkers were undetectable. TS protein expression was detected in by SRM in 2 patients and by IHC in 32 patients (tumor staining>1); however, TS IHC was not predictive of outcome (PFS-HR ratio = 1.06). Patients whose tumors expressed low levels of GARFT protein ($\leq$900 amol/μg; n=7) had statistically significantly longer median PFS than those whose tumors expressed high levels of GARFT (>900 amol/μg; n=30) (40.6 vs. 11.4 months; p = 0.014). Patients with high FRalpha protein expression (>1510 amol/μg, n=9) had significantly longer median PFS than those with low FRalpha expression ($\leq$1510 amol/μg; n=28) (>50 vs. 11.4 months; p= 0.021). Moreover, the 23 patients with both high GARFT expression (>900 amol/μg) and low FRalpha expression ($\leq$1510 amol/μg) faired considerably worse than the remainder of patients (median PFS 10.1 vs. 40.6 months; p=0.0003).

Conclusion: Multiplexed mass spectrometry-based proteomics offers a feasible and promising approach for tumor biomarker profiling and quantification to predict therapeutic response. Of note, our results show that FRalpha and GARFT protein expression may be predictive of response to pemetrexed-based treatment in patients with non-sq NSCLC. Further investigation is needed to validate the utility of these biomarkers for guiding personalized treatment decisions in clinical practice.

Deregulated SOX2 drives dysplasia in a novel 3D organotypic model of bronchial dysplasia

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Introduction and objectives: Squamous lung cancer (SQC) is a devastating disease for which the currently available treatments are poorly effective. There are no licensed targeted therapeutic agents. Improving the early detection and chemoprevention of SQC, and identifying novel and tractable therapeutic targets are key challenges to improving outcomes.

The development of model systems that recapitulate the human disease will facilitate the development of new therapeutic/chemopreventive agents and new insights into the pathobiology of SQC.

SOX2 amplification is a frequent genetic alteration in squamous lung cancer. We have shown that amplification and overexpression of SOX2 is an early and consistent event in the pathogenesis of this disease.

Our hypothesis is that deregulated expression of SOX2 is a key event in the initiation of bronchial dysplasia/squamous carcinogenesis.

Our aims are:

1) To develop a novel 3D in vitro model of human bronchial dysplasia that recapitulates the molecular and phenotypic characteristics of the in vivo disease

2) To test the hypothesis that SOX2 deregulation is a key initiating event in the pathogenesis of bronchial dysplasia

3) To define the functional impact of SOX2 deregulation

Methods: We use lentiviral transduction and Cas9/CRISPR editing genome to genetically modify immortalized human bronchial epithelial cells (HBECs); thus creating a suite of cells with complex genotypes, recapitulating the molecular lesions seen in the human disease. Genetically manipulated HBECs are grown at the air-liquid interface on a stromal equivalent with embedded pulmonary fibroblasts – an organotypic system (OTC). We use an inducible construct so that SOX2 is only deregulated in HBECs that are already confluent at the air-liquid interface. OTCs are processed for downstream analyses including immunohistochemistry, cell cycle analyses, RNA- Seq and ChIP-Seq.

Results: We have developed a novel model that recapitulates human bronchial dysplasia. SOX2 deregulation does not impact upon cell cycle progression or cell population expansion in standard tissue culture conditions. However, in the organotypic system described above, overexpression of SOX2 in confluent HBECs at the air-liquid interface initiates focal dysplastic outgrowths from the monolayer that exhibit histological characteristics of high-grade human bronchial dysplasia.

Combining SOX2 activation with the loss of TP53 markedly potentiates the dysplastic phenotype, recapitulating the likely in vivo sequence of molecular events. SOX2 overexpression alters key cell signaling pathways (MAPK and PI3K/AKT) in a pattern reflecting those seen in squamous lung cancer.

Recent RNA- and CHiP-Seq analyses suggest SOX2 regulates a series of target genes implicated in cell migration and metastasis, key hallmarks of malignant progression. These results are currently being validated and functional validation planned.

Conclusions: In the appropriate molecular and microenvironmental context acute deregulation of SOX2 initiates and drives bronchial dysplasia in immortalized human bronchial epithelial cells. This work is consistent with many others in which the microenvironment has been shown to be as critical as cell intrinsic pathways in the development of cancer.

This model system is well suited for screening novel therapeutics and chemoprevention agents as well advancing our understanding of the pathobiology of SQC. Further work is underway to evaluate the functional impact of SOX2 deregulation in this short-term model of human bronchial dysplasia.

ELF3 amplification at 1q32.1 promotes SMAD4-independent tumorigenesis

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Background: E74-like factor 3 (ELF3) is a member of the ETS family of transcription factors that was recently identified as an important target of SMAD4 in a mouse model of lung tumorigenesis. ELF3 is also a described oncogene in breast and prostate cancer; however, little is known about the function of ELF3 in human lung cancer and the dependence of ELF3 overexpression on SMAD4 loss. Here we investigate this dependence, as well as alternative genetic and epigenetic mechanisms of ELF3 overexpression in two independent cohorts of lung adenocarcinoma (AC).

Methods: DNA copy number, promoter methylation, and mRNA expression data was queried in two cohorts of lung AC: BC Cancer Research Centre (n=83) and The Cancer Genome Atlas (TCGA) (n=551). Mutation information was only available for the TCGA cohort. GISTIC 2.0 was used to determine significantly focally amplified regions from segmentation data. Stable shRNA knock-down of ELF3 was performed in a panel of AC cell lines with high ELF3 expression. Similarly, expression of ELF3 in HBECs was mediated by lentiviral delivery of an overexpression vector. Cell viability was assessed by MTT assay, while proliferation was assessed by BrdU assay and colony formation assay. Tumor growth was monitored *in vivo* following flank injections of isogenic cell lines into NOD-SCID mice. ELF3 cellular localization in lung AC cell lines was assessed by western blot and immunofluorescence, and in tumors by immunohistochemistry. Associations with clinical features were determined by Fisher's tests, while survival analyses were performed using the log-rank method.

Results: ELF3 overexpression occurred in over two-thirds of lung adenocarcinomas and was not dependent on SMAD4 copy number loss or underexpression. In fact, tumors with high expression of ELF3 were more likely to recur than those with SMAD4 loss indicating divergent biological functions. ELF3 overexpression was frequently selected-for at the DNA